Cryptogamie, Mycol. 1996, 17 (1): 39-46

ISOLATION OF FUNGI FROM HUMAN HAIR SAMPLES COLLECTED IN EL-BAHRIN AND THE ANTIFUNGAL ACTIVITY OF VARIOUS SHAMPOOS

A.H.M. El-SAID

Botany Department, Faculty of Science, Qena University, Egypt.

ABSTRACT — Twenty-eight species and 3 varieties representing 10 genera of keratinophilic and saprophytic fungi were isolated from 25 healthy human hair samples collected from El-Bahrin on two isolation media. Members of *Aspergillus*, *Penicillium* and *Chrysosporium* were the most predominant on the hair samples.

The antifungal effect of twelve kinds of shampoos were tested on 10 strains of keratinophilic and saprophytic fungi. Eleven of these shampoos were found to be effective against all the tested fungi. Keratinophilic fungi were more sensitive than saprophytic fungi.

KEY WORDS: Keratinophilic fungi, saprophytic fungi, antimycotic activity shampoo.

INTRODUCTION

The hair of human are contaminated by numerous fungi, some of which are apparently opportunistic pathogens or allergens. There are a few studies about anthropophilic dermatophytes in healthy persons (Moharram *et al.*, 1988; Zaror & Aliaga, 1990). This may be due to the fact that the fungus presence in some carriers is non-apparent, the methods of detection have not been appropriate or that both human and lower animals have an innate resistance to invasion by these fungi (Zaror & Aliaga, 1990). The presence of anthropophilic dermatophytes in healthy persons makes it necessary to continue the study of these fungi for a better knowledge of their life-cycle and ecology and for an adequate control by preventive measures and/or therapeutic action, when necessery.

The present study was carried out in order to detect dermatophytes, keratinophilic and saprophytic fungi in the hair of healthy persons from El-Bahrin. Also, the antifungal activity of some cosmetics, shampoos and oils on common fungi was studied.

MATERIALS AND METHODS

Twenty-five samples of human hair (25-40 years old) were collected from healthy persons in El-Bahrin by using sterile scissor. These samples were placed in sterile clean plastic bags and transferred immediately to the laboratory to screen for their fungal contents as follows:

Keratinophilic fungi:

These fungi were isolated by using a soil hair-baiting technique. The sandy-clay soil was double sterilized by autoclaving at 120°C for 30 min. The hair samples were placed on the surface of the sterile soil moistened with sterile distilled water (25-40 % moisture content) in plates (2 plates for each sample). The plates were incubated at room temperature for 10 weeks and remoistened whenever necessary during the incubation time. After incubation period the moulds which appeared on the bait hairs were transferred to the surface of plates containing sabouraud's dextrose agar medium (Moss & McQuown, 1969) supplemented with cycloheximide (0.05 mg/ml) and chloramphenicol (40 units). The plates were incubated at 25°C for 2 weeks and the developing fungi were identified (Carmichael, 1962; Rohd & Hartmann, 1980; Van Oorschot, 1980) and counted per 10 hairs in each sample. The relative importance values (RIV) were calculated for each fungal species (Shearer & Webster, 1985; Ali-Shtayeh *et al.*, 1988).

Saprophytic fungi:

Saprophytic fungi associated with human hairs were isolated by using the dilution-plate method (Johnson & Curl, 1972). Two g of hair sample is placed in 250 ml Erlenmeyer flask. Sterile distilled water is add to the hair samples so that a total volume of 200 ml is reached. The flask containing the suspension is shaken on a mechanical shaker for 5 min. Ten ml of this suspension are immediately drawn (while in motion) using sterile Menzies (1957) dipper and tranferred immediately through a known volume of sterile water blank untill the desired final dilution is reached which supports a total of about 25-40 colonies per dish. The suspension is shaken by hand for few minutes. One ml of the desired dilution is transferred aseptically into a sterile petri-dishes and 12 to 15 ml of an appropriate agar medium, cooled to just above the solidifying temperature, are added to dishes. The dishes are rotated by hand in a broad swirling motion. So that the suspension is dispersed in the agar. Modified Czapek's agar medium, in which 3% sucrose was substituted with 1% glucose, was used. Two plates were used for each hair sample and incubated at 28° C for 1-2 weeks. The appearing fungi were counted, identified (purely morphological, based on macro — and microscopic characteristics, Raper & Fennell, 1965; Pitt, 1985; Domsch *et al.*, 1980) and calculated per g hair.

Antimycotic activity of different types of cosmetics:

Twelve different kinds of creams, shampoos and oil, commonly used for cleaning the human hairs were tested for their antifungal activity. Herbal essence with natural protein, Aloe Eva, Polytar, Oil, Lactuel vitamin care, Panthenol, Glemo

coconut, Palm olive and Egg lactuel were manufactured in Egypt; Pert plus in Switzerland; Soft in Germany; and Selsun blue in the U.S.A. The test organisms were cultured on 20 ml sabouraud's dextrose agar medium. Cultures were incubated at 28°C for 7 days. Using sterile cork borer 10 mm diameter, 3 discs were cut to inoculate 50 ml sterile liquid water (in 250 ml Erlenmeyer conical flasks) to obtain the spore suspension. Twenty ml of sterilized sabouraud's dextrose agar medium were poured into a sterilized petri-dish containing 1 ml spore suspension of each test organism. After hardening 3 discs (3 mm) of filter paper (Whatman No. 3), fully saturated individually by the tested cosmetic, were placed on the agar surface of the plates. On another plates, three discs without cosmetic were placed on the agar surface as a control. The plates were incubated for 1-2 weeks at 28°C. The diameter of the inhibition zone around the disc was measured using the ruler.

RESULTS AND DISCUSSION

Keratinophilic fungi:

Six species belonging to 2 genera were identified from the hair samples using the soil hair-baiting technique on sabouraud's dextrose agar medium at 25° C (Table 1). Most of these fungi are already known as colonizers of bait hair (Filipello-Marchisio & Luppi Mosca, 1982; Polonelli *et al.* 1982; Mercantini *et al.*, 1983, 1986; Ali-Shtayeh & Arada, 1985; Nigam & Kushwaha, 1989; Roig *et al.*, 1989).

Chrysosporium was the first keratinophilic fungi isolated from the persons hair and were present in 96% of the hair represented 86.6% of total isolates with RIV of 182.6. De Vroey (1976) mentioned that *chrysosporium* species are occasionally isolated in the clinical laboratory from skin, hair or nails. From the genus, 4 species were identified of which *C. tropicum* and *C. keratinophilum* were prevalent. They occurred in 64% and 52% of the samples comprising 34.9% and 23.3% of total fungi, respectively. Moharram *et al.* (1988) isolated the above two species from human hair, in Egypt, in low (21%) and rare (5%) frequency. Filipello-Marchisio (1986) studied the keratinolytic ability of some fungal isolates and showed that *C. tropicum* and *C. keratinophilum* came among the species which were the most active keratinolytically. These two species were also, predominant in floors of Roman primary and secondary schools (Mercantini *et al.*, 1983, 1986) and the sands of a box for childrens play (Filipello-Marchisio & Luppi Mosca, 1982). *C. asperatum* (5.8% of the isolates) and *C. indicum* (6.4%) were less common (Table 1).

Trichophyton (2 species) was isolated in rare frequency of occurrence. It was encountered in 3 samples (out of 29) contributing 13.4% of total isolates which have RIV of 25.3. Of the genus *T. rubrum* (4.7% of total isolates) and *T. terrestre* (8.7%) were isolated (Table 1). Mariat *et al.* (1967) reported that 15% of African immigrant population in the France were carriers of scalp dermatophytes. They isolated *T. rubrum* from 3% of the samples. But, Lopez-Martinez & Rivera Liona (1984) isolated *T. rubrum* from 28 % of the persons. While Sinski & Kelley (1991) isolated *T. terrestre* in 4 times from 45 cities and 1 state in the USA.

Genera & species	TI	NCI & OR	RIV
Chrysosporium	149	24H	182.6
C.asperatum Carmichael	10	2R	13.8
C.indicum (Randhawa & Sandhu) Gary	11	5L	26.3
C.keratinophilum (D.Frey) Carmichael	40	13H	75.2
C.tropicum Carmichael	60	16H	98.8
Chrysosporium sp.	28	6L	40.2
Trichophyton	23	3R	25.3
T. rubrum (Castellani) Sabouraud	8	2R	12.6
T. terrestre Durie & Frey	15	2R	16.7
Total isolates	172		
Number of genera	2		
Number of species	6		

Table 1: Total isolates (T I, calculated per 250 hair fragments in all samples), number of cases of isolation (NCI, out of 25), occurrence remarks (OR) and relative importance values (RIV) of keratinophilic fungi recovered from 25 human hair samples at 25°C .
Occurrence remarks (OR): H = high occurrence, between 13-25 cases (out of 25); M = moderate occurrence, between 7-12 cases; L = low occurrence, between 4-6 cases; R = rare occurrence, between 1-3 cases

Saprophytic fungi:

A total of 10576 colonies representing twenty-two species and 3 varieties in addition to 8 genera were isolated from human hair samples on plates of 1% glucose-Czapek's agar at 28°C. Many of these fungi were isolated from human skin of patients with no evidence of clinical lesions (Lopez-Martinez *et al.*, 1978).

Aspergillus was the more common genus, found in all samples having 77.9% of total isolates. It was represented by 9 species and 1 variety of which *A. flavus, A. fumigatus* and *A. niger* were of highest occurrence. They emerged in 100, 100 and 84% of the samples matching 30.2, 22.9 and 14.2% of total fungi, respectively. *A. ochraceus* (2.8% of total fungi) was moderately encountered and *A. terreus* (4.4%) was of low incidence. The remaining *Aspergillus* species were rare on human hair (Table 2). These results were greatly similar to those obtained by Moharram *et al.* (1988). They noticed that *Aspergillus* (10 species + 1 variety) was the first most dominant fungi on human hairs in Egypt. Also, Arreeza & Urrestarazu (1988) isolated *A. niger* and *A. terreus* from patients with a possible diagnosis of onychomycosis.

Penicillium (6 species) and *Emericella* (1 + 2 varieties) were not as common as the *Aspergillus*. They were isolated in high and moderate occurrence, encountered in 72 and 44% of the hair and 11.3 and 6.8% of total fungi, respectively. From the two genera *P. chrysogenum, P. oxalicum, E. nidulans* and *E. nidulans* var. *lata* were the most prevalent species. The remaining *Penicillium* and *Emericella* species were less frequent (Table 2). Aho *et al.* (1990) showed that *Penicillium* species known to infect humans and *P. chrysogenum, P. citrinum* and *P. purpurogenum* cause penicilliosis. Also, they noticed that, despite the wide spread distribution and prevalence of the 150 to 214 (Pitt,

Genera & species	ATC	% C	NCI & OR
Alternaria alternata (Fries) Keissler	93	0.9	3R
Aspergillus	8235	77.9	25H
A. aureolatus Munt. , Cvet. & Bata	106	1.1	3R
A. flavus Link	3193	30.2	25H
A. fumigatus Fresenius	2425	22.9	25H
A. niger Van Tieghem	1500	14.2	21H
A. ochraceus Wilhelm	300	2.8	7M
A. sydowii (Bain.& Sart.) Thom & Church	56	0.5	2R
A. terreus Thom	462	4.4	4L
A. terreus var. *africans* Fennell & Raper	87	0.8	2R
A. ustus Fennell & Raper	50	0.5	1R
A. versicolor (Vuill.) Tiraboschi	56	0.5	2R
Chaetomium globosum Kunze	87	0.8	2R
Emericella	716	6.8	11M
E. nidulans (Eidam) Vuillemin	231	2.2	7M
E. nidulans var. *dentata* Sandhu & Sandhu	143	1.4	3R
E. nidulans var. *lata* (Thom & Raper) Subram.	342	3.2	7M
Mucor circinelloides Van Tieghem	31	0.3	1R
Penicillium	1191	11.3	18H
P. aurantiogriseum Dierckx	93	0.9	2R
P. chrysogenum Thom	512	4.8	12M
P. citrinum Thom	112	1.1	3R
P. corylophilum Dierckx	87	0.8	3R
P. oxalicum Currie & Thom	225	2.1	7M
P. puberulum Bainier	162	1.6	4L
Rhizopus stolonifer (Ehrenb.) Lind.	37	0.3	1R
Sterile mycelia (white & dark colour)	112	1.1	3R
Ulocladium	74	0.6	1R
U. botrytis Preuss	37	0.3	1R
U. chartarum (Preuss) Simmons	37	0.3	1R
Gross total count	10576		
Number of genera	8		
Number of species	22+ 3 var.		

Table 2: Average total count (ATC, calculated per ▪ hair), percentage count (% C calculated per total fungi), number of cases of isolation (NCI, out of 25 samples) and occurrence remarks (OR) of fungal genera and species recovered from 5 human hair samples on 1% glucose-Czapek's agar at 28°C.

Occurrence remarks (OR): H =high occurrence, from 13-25 (out of 25); M= moderate occurrence, from 7-12; L= low occurrence, from 4-6; R= rare occurrence, from1-3 cases.

Fungus	Herbal essence with natural protein	Soft	AloeEva	Polytar	Pert plus	Oil	Lactuel vitamin care	Panthenol	Glemo coconut	Palmolive	Egg lactuel	Selsun blue
Aspergillus flavus	15	33	23	20	21	20	19	13	9	11	13	22
A. fumigatus	20	31	22	21	28	24	24	20	22	16	19	20
A. niger	17	26	23	20	22	24	25	16	11	12	16	18
Chrysosporium asperatum	36	0	42	40	44	46	30	41	35	35	34	47
C. indicum	35	0	56	44	48	45	50	39	37	43	49	61
C. keratinophilum	31	0	45	37	50	44	34	24	32	33	24	34
C. tropicum	33	0	40	38	36	41	31	27	32	29	25	49
Chrysosporium sp.	44	0	48	39	40	42	41	34	35	37	38	28
Trichophyton rubrum	36	0	34	35	27	33	32	28	30	31	34	40
T. terrestre	37	0	41	37	38	44	34	33	31	31	27	35

Table 3: Diameter (in mm) of the inhibition zone induced by the different types of cosmetics on some selected isolates of keratinophilic and saprophytic fungi.

1979; Ramirez, 1982) recognized species of *Penicillium*,only eight have been unequivocally known as agents of infections disease.

The remaining 6 species were encountered in rare occurrence and were representing collectivelly about 2.9% of total fungi (Table 2).

Effect of shampoos on fungi:

The fungistatic nature of shampoos and oils was shown in table (3). The results indicated that eleven, out of twelve, of shampoo proved to be effective against all the tested fungi. Saprophytic fungi represented by *Aspergillus flavus*, *A. fumigatus* and *A. niger* were the most resistance fungi to shampoos while, the keratinophilic fungi represented by *Chrysosporium* and *Trichophyton* species were sensitive. This was in agreament with the results obtainted by Moharram *et al.* (1988). They reported that *Chrysosporium* isolates were the most sensitive fungi to shampoos. Aloe eva, pert plus and lacutel vitamin care were highly effective against all test organisms. The shampoo glemo coconut and palmolive exhibited moderate suppressive effect against the majority isolates and were weak against few isolates. Soft shampoo showed no inhibition effect against keratinophilic fungi and weak against saprophytic one. The remaining types of creams and oils showed inconsistance effect on the fungi tested (Table 3). In this respect, Garg *et al.* (1985) reported that linoleic acid completely suppressed the growth of *Trichophyton ajelloi*, *Ctenomyces serratus* and *Microsporum gypseum*. Also, Moharram *et al* (1988) noticed that among twelve types of creams, three of them were highly effective against all fungi tested (Lanolin, Relax bath and herb-green). But, the shampoo balsam exhibited weak suppressive effect against few isolates and was inactive against the majority of isolates.

Acknowledgement

The auther is deeply indebted to Prof. S. I. I. Abdel-Hafez (Professor of Microbiology, Botany Department, Assiut University) for his kind help.

REFERENCES

AHO R., WESTERLING B., AJELLO L., PADHYE A. A. & SAMSON R. A., 1990 — Avian Penicilliosis caused by *Penicillium griseofulvum* in a captive toucanet. *Journal of medical and veterinary mycology* 28: 349-354.

ALI-SHTAYEH M. S. & ARDA H. M., 1985 — Incidence of dermatophytosis in Jordan with special reference to tinea capitis. *Mycopathologia* 92: 59-62.

ALI-SHTAYEH M. S., ARDA H. M., HASSOUNA M. & SHAHEEN S. F., 1988 — Keratinophilic fungi on the hair of goats from the West Bank of Jordan. *Mycopathologia* 104: 103-108.

ARREAZA F. & URRESTARAZU M. I., 1988 — Study of the mycotic and bacterial flora in patients with nail lesions. *Medicina cutanea Ibero-Latino-Americana* 16: 285-290.

CARMICHAEL J. W., 1962 — *Chrysosporium* and some other Aleruiosporic Hypomycetes. *Canadian Journal of Botany* 40: 1137-1172 .

DE VROEY C., 1976 — Ascomycetes isolated from skin lesions in man. *Bulletin de la société française de mycologie médicale* 5: 161-164.

DOMSCH K. H., GAMS W. & ANDERSON T., 1980 — *Compendium of soil fungi*, Academic Press, London. 859p.

FILIPELLO-MARCHISIO V. & LUPPI MOSCA A. M., 1982 — Mycological analysis of the sands of a box for children's play. *Mycopathologia* 80: 43-54.

FILIPELLO-MARCHISIO V., 1986 — Keratinolytic and keratinophilic fungi of children's sandpits in the city of Turan. *Mycopathologia* 94: 163-172.

GARG A. P., SMITH S. N. & PUGH J. F., 1985 — Inhibition of growth of keratinophilic fungi by oils and fatty acids. *Transaction of the british mycological society* 85: 367-370.

JOHNSON L. F. & CURL E. A., 1972 — *Methods for research on ecology of soil-borne pathogen.* Burgess Publ. Co. Minneapolis: 247.

LOPEZ-MARTINEZ R., MARIAT F. & DOMINGUEZ L., 1978 — Aistamiento de dermatofitos de piel calbelluda sana. *Boletin de la sociedad méxicana de micologia* 12: 103-109.

LOPEZ-MARTINEZ R. & RIVERA LLONA M., 1984 — Investigation de dermatofitos en La piel sana de diversas regions corporales. *Revista latinoamerica de microbiologia* 25: 365-369.

MARIAT F., ADAMS-CAMPOS C., GENTILINI M. & GLAXOTTE P., 1967 — Presence de dermatophytes chez L'homme en l'absence de lesions cliniques. *Bulletin de la société française de dermatologie et de syphiligraphie* 74: 724-729.

MENZIES J. D., 1957 — A dipper technique for serial dilution of soil for microbial analysis. *Proceedings of the soil science society of America* 21: 660 .

MERCANTINI R., MARSELLA R., LAMBIASE L. & FULVI F., 1983 — Isolation of keratinophilic fungi from floors in Roman primary schools. *Mycopathologia* 82: 115-120.

MERCANTINI R., MARSELLA R., LAMBIASE L. & BELARDI M., 1986 — Isolation of keratinophilic fungi from floors in Roman kindergarten and secondary schools. *Mycopathologia* 94: 109-115.

MOHARRAM A. M., ABDEL-GAWAD K. M. & EL-MARAGHY S. S. M., 1988 — Ecological and physiological studies on fungi associated with human hair. *Folia Microbiologica* 33: 363-371.

MOSS E. S. & MCQUOWN A. L., 1969 — *Atlas of Medical Mycology, 3rd ed.* Williams and Wilkins, Baltimore. 366 p.

NIGAM N. & KUSHWAHA R. K. S., 1989 — Some reports on keratinophilic fungi. *Current Science* 58: 1374.

PITT J. I., 1979 — *The genus penicillium and its teleomorphic states Eupenicillium and Talaromyces.* Academic Press, London. 634 p.

PITT J. I., 1985. *A laboratory guide to common Penicillium species.* Commonwealth scientific and industrial research organization division of food research . 184 p.

POLONELLI L., GARCOVICH A. & MORACE G., 1982 — Dermatophytes carriers among school children. *Mykosen* 25: 254-257.

RAMIREZ C., 1982 — *Manual and atlas of penicilla.* New York, Elsevier Biomedical Press. 847 p.

RAPER K. B. & FENNELL D. J., 1965 — *The genus Aspergillus.* Williams and Wilkins Baltimore, U.S.A. 686 p.

RODHE B. & HARTMANN G., 1980 — *Introducing mycology by examples.* Presented by Schenng Ahtiencesellschaft. P.136 .

ROIG A. M., BONASTRE M., GARCIA PEREZ A. & TORRES RODRIGUEZ J. M., 1989 — Healthy carriers of dermatophytes among children population. *Revista ibérica de Micologia*, 5: 118.

SHEARER C. A. & WEBSTER J., 1985 — Aquatic hyphomycetes communities in the river Teign. III-Comparison of sampling techniques. *Transaction of the british mycological society*, 84: 509-518.

SINSKI J. T. & KELLEY L. M., 1991 — A survey of dermatophytes from human patients in the United States from 1985 to 1987. *Mycopathologia* 114: 117-126.

VAN OORCSHOT C. A. L., 1980 — A revision of *Chrysosporium* and allied genera. *Studies in mycology* 20: 1-87 .

ZAROR L. & ALIAGA X., 1990 — Dermatophytes in healthy chilians. *Mycoses* 33: 95-98.